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BUOYANCY MECHANISMS LIMIT PRESERVATION OF COLEOID CEPHALOPOD SOFT TISSUES IN MESOZOIC LAGERSTÄTTEN

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Abstract: Coleoid cephalopods are characterized by internalization of their shell, and are divided into the ten-armed Decabrachia (squids and cuttlefish) and the eight-armed Vampyropoda (octopuses and vampire squid). They have a rich fossil record predominantly of the limited biomineralized skeletal elements they possess: arm hooks, statoliths, mouthparts (the buccal mass) and internal shell (gladius or pen), although exquisitely preserved soft tissue coleoids are known from several Lagerstätten worldwide. Recent studies have shown that although morphological similarities between extant decabrachian gladii and fossil examples exist, no known examples of fossil decabrachians are currently known. However, molecular clock data and phylogenetic bracketing suggest that they should be present in Lagerstätten that are rich in vampyropod soft tissue fossils (i.e. Hâkel and Hâdjoula Lagerstätten, Cretaceous, Lebanon). We propose that a hitherto unknown taphonomic bias pertaining to the differing methods of buoyancy control within coleoid groups limits preservation potential. Both negatively and neutrally buoyant decabrachians use chemical buoyancy control (ammonia) whereas vampyropods do not. In the event of rapid burial in an environment conducive to exceptional preservation, ammonia dramatically decreases the ability of the decabrachian carcass to generate the required pH for authigenic calcium phosphate replacement, limiting its preservation potential. Moreover, the greater surface area and comparatively fragile dermis further decrease the potential for fossilization. This taphonomic bias may have contributed to the lack of preserved labile soft-tissues in other cephalopods groups such as the ammonoids.

Key words: coleoid, cephalopod, buoyancy, calcium phosphate, taphonomy, bias.

Cephalopods have a rich fossil history, illustrating the evolution from shelled molluscs into nektonic predators, dominated today by the coleoids (octopods, squid and cuttlefish) (Kröger *et al.* 2011). The tempo and mode of cephalopod evolution can be understood through studying the fossil record in a phylogenetic framework; a key trend in coleoid evolution is the reduction of the protective shell through internalization and reduction in several clades (Lindgren *et al.* 2012). Coleoids are separated into two main groups based on the number of arms: the ten-armed Decabrachia, represented by squids and cuttlefish, and the eight-armed Vampyropoda consisting of the Octopoda and the vampire squid *Vampyroteuthis infernalis* (Vampyromorpha) (Kröger *et al.* 2011; Sutton *et al.* 2015; Donovan & Fuchs 2016).

An issue for palaeontologists is that diagnostic soft tissues are rarely preserved, making positive identification of ancestral coleoids difficult. Despite their minimal biomineralized tissues, the coleoid fossil record is surprisingly rich with gladii, mouthparts (the buccal mass) (Tanabe *et al.* 2015), arm hooks (Engeser & Clarke 1988; Harzhauser 1999) and statoliths (the mineralized part of the balance sensory receptor; Hart *et al.* 2013; Klug *et al.* 2016; Neige *et al.* 2016) frequently found fossilized worldwide. Conversely, coleoid soft tissues are preserved in only a handful of Mesozoic and Cenozoic Lagerstätten (Fig. 1). There is, nevertheless, significant difficulty in inferring taxonomic position using isolated mouthparts, arm hooks or statoliths (see Klug *et al.* 2005; Hart *et al.* 2015) meaning that fossil gladii are often the primary means of

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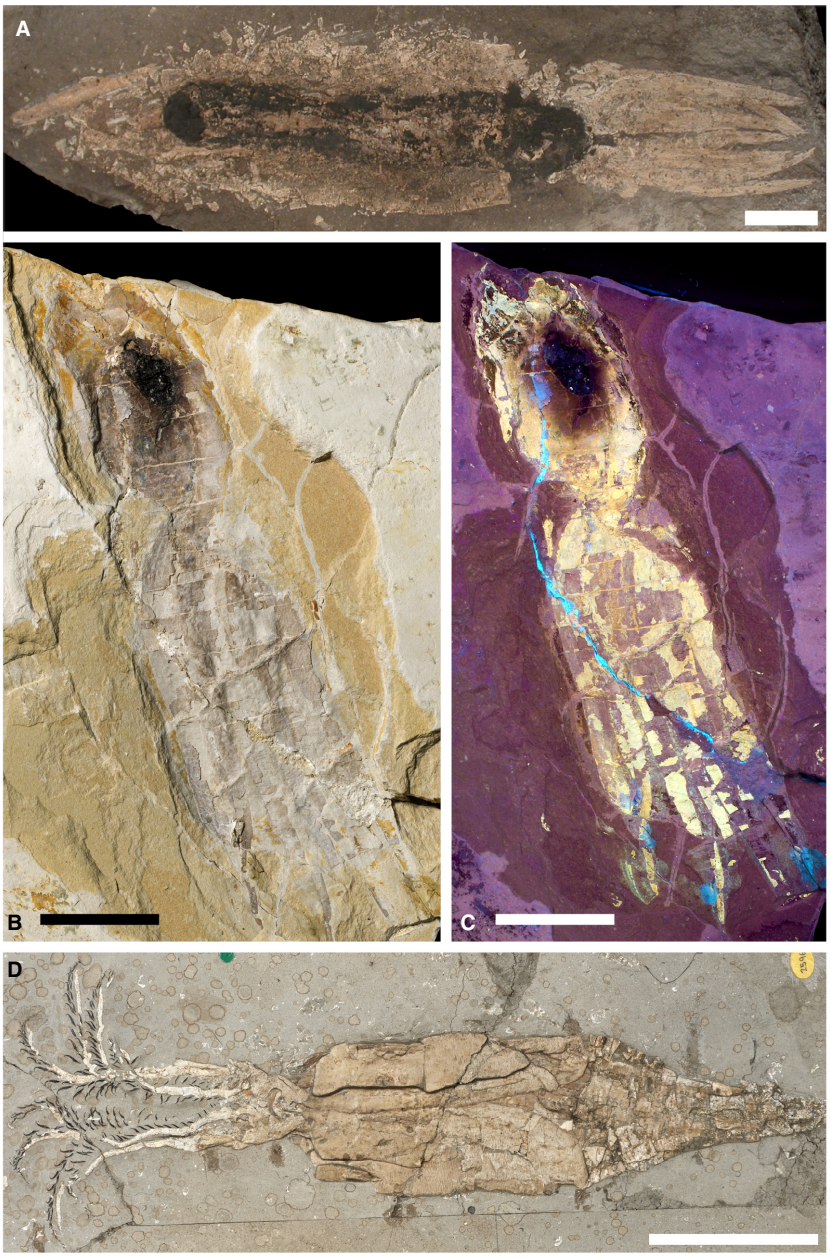

FIG. 1. Examples of stem and crown fossil vampyropods and fossil stem decabrachians. A, stem vampyropod *Loligosepia aalensis*, Bachhausen (Neumarkt), Germany, lower Toarcian, P60140b; LWL-Museum für Naturkunde, Münster (photo courtesy of Dirk Fuchs). B, crown vampyropod *Keuppia* sp., Cretaceous, Hâkel, Lebanon, NHMUK CC578A. C, *Keuppia* sp. under uv light showing phosphatized tissues which fluoresce brightly against the rock matrix. D, stem decabrachian, *Belemnotheutis antiquus*, Christian Malford, Wiltshire, England, Callovian Oxford Clay, NHMUK 25966. (Photos B–D courtesy of Jonathan Jackson, NHMUK). All scale bars represent 5 cm. Colour online.

taxonomic identification (e.g. Naef 1922). Since the 1800s, most fossil coleoids have been described as fossil teuthids (squids) due to the considerable morphological similarity with extant decabrachian species (Jeletzky 1966) coupled with the previous consensus that only squid possessed a well developed gladius (Donovan & Toll 1988).

Recent reassessment of Mesozoic gladii has questioned the validity of the diversity, taxonomy and phylogeny of the fossil teuthids (teuthids refer to gladius-bearing decabrachian coleoids, thus excluding cuttlefish and spirulids and probably reflecting a polyphetic grouping). In 1986, German palaeontologists Bandel and Leich revised three fossil teuthids that preserved soft tissues from the Solnhofen Lagerstätte. They established that no fossils from this locality exhibited more than eight arms and, furthermore, they identified other vampyromorph characteristics such as cirri and umbrella-like webbing between the arms (Bandel & Leich 1986). Subsequently, they reclassified three fossil coleoids from the ten-armed Decabrachia to the eight-armed Vampyropoda. Since then, better understanding of the reduced internal shell of *Vampyroteuthis* and the cirrate octopods (Gibson *et al.* 2006) combined with the increased awareness of the superficial morphological similarities between fossil and extant teuthid gladii has led to the re-examination of many other fossil coleoid

species. Presently, no examples of arm crowns with ten unequivocal arms have been identified (Bandel & Leich 1986; Donovan *et al.* 2003; Fuchs *et al.* 2003, 2007, 2009, 2013*b*; Fuchs 2007; Fuchs & Weis 2008, 2009, 2010; Fuchs & Larson 2011*a*, *b*; Arkhipkin *et al.* 2012; Klug *et al.* 2015; Donovan & Fuchs 2016) except in belemnoids (stem Decabrachia). The mass reclassification of fossil teuthids in the Vampyropoda has been termed the 'Vampyropoda hypothesis' and indicates a complete absence of decabrachian teuthid coleoids in the fossil record (Fuchs & Larson 2011*a*; Fuchs *et al.* 2015*a*). The most recent phylogenetic work using both extant and fossil data corroborates the Vampyropoda hypothesis (Sutton *et al.* 2015).

The most exceptional coleoid fossils with preserved soft tissues are found in the Late Cretaceous (late Cenomanian) Lagerstätten of Hâkel and Hâdjoula, where examples of eight-armed Vampyropoda (Fuchs 2007; Fuchs & Weis 2009; Fuchs *et al.* 2009, 2015*b*; Fuchs & Larson 2011*a*, *b*), such as *Keuppia*, *Styleoctopus* and *Palaeoctopus*, are discovered relatively frequently with phosphatized soft tissues including arm crowns, limbs, suckers, ink sacs, gills, eye capsules and respiratory, excretory and circulatory systems (Fuchs *et al.* 2015*b*). Despite palaeoenvironmental conditions suitable for the preservation of coleoid labile tissues, there is no evidence of teuthids (Fuchs 2007). Recent studies of molecular clock data surmise that teuthids and the other decabrachians (i.e. cuttlefish) diverged around 150 (±30) Ma and therefore co-existed with Vampyropoda during the late Mesozoic (Fig. 2) (Kröger *et al.* 2011; Warnke *et al.* 2011). Indeed, fossil cuttlebones (the mineralized and chambered

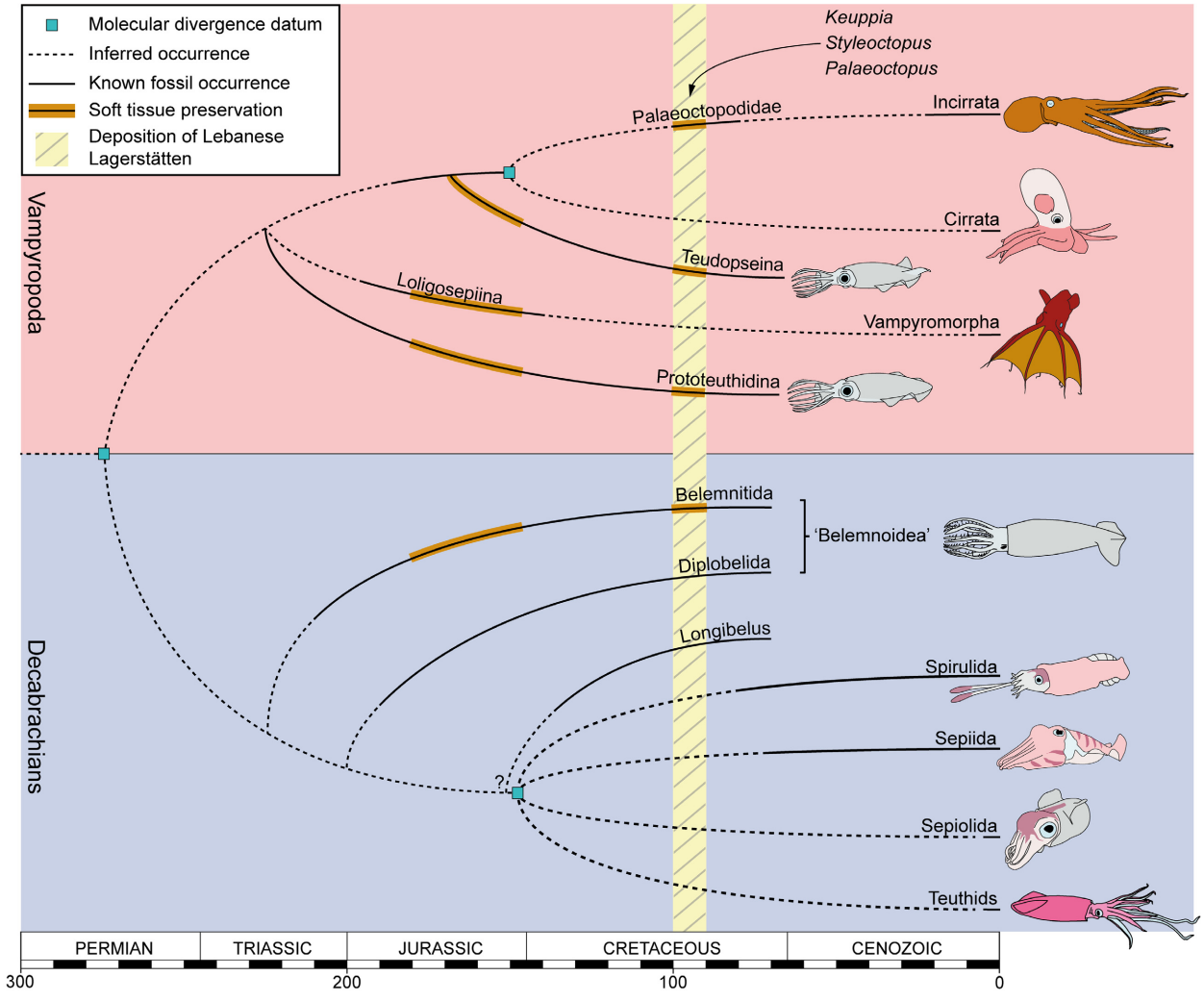

FIG. 2. Phylogeny of the major coleoid groups. Solid lines indicate known fossil occurrences of coleoid tissues with highlighted lines representing soft tissue preservation. Squares indicate molecular divergence datum (taken from Kröger *et al.* 2011). The depositional time period of the mid-Cretaceous Lebanese Lagerstätten where decabrachian fossils are not found is marked. Colour online.

shell of the cuttlefish) (Hewitt & Jagt 1999; Fuchs *et al.* 2012*b*), decabrachian jaws (Tanabe *et al.* 2015) and the internal shell of a type of deep-water squid (e.g. *Spirula spirula*) (Fuchs *et al.* 2012*a*, 2013*a*) have been found in other Cretaceous sediments. Recent work has proposed that teuthids diverged from a belemnoid ancestor (*Longibelus* gen. nov.) during the Early Cretaceous (Fuchs *et al.* 2013*a*) and probably radiated quickly, refilling niches left by the gradual extinction of the belemnites (Iba *et al.* 2011). These temporal constraints suggest that the Decabrachia were diverse during the Cretaceous, but there are no unequivocal examples of body fossils despite phylogenetic bracketing predicting that teuthid decabrachians should have been present at this stage (Fig. 2).

Exceptional preservation of coleoid cephalopods

The preservation of coleoid soft tissues is well documented (e.g. Allison 1988*b*; Briggs 2003; Wilby *et al.* 2004; Fuchs & Larson 2011*a*) (Fig. 1). In order for non-biomineralized coleoid soft tissues to preserve, diagenetic mineralization must occur. The replacement of labile tissues by varying diagenetic minerals yields a range of histological fidelity of morphological characters (Allison 1988*b*; Briggs & Kear 1993; Briggs & Wilby 1996; Wilby & Briggs 1997). Calcium phosphate is known to replicate fine scale morphological detail, preserving to the cellular level (Allison 1988*b*; Briggs & Kear 1993; Briggs & Wilby 1996; Wilby & Briggs 1997). However, under 'normal' marine conditions, calcium carbonate preferentially precipitates (Briggs & Wilby 1996; McNamara *et al.* 2009). The dominant control of phosphatic preservation is the pH of the environment surrounding the organism carcass (Briggs & Wilby 1996). Decreasing pH is a noted phenomenon within decaying organic tissue due to acidic by-products, such as sulphuric acid (H_2SO_4), CO_2(aq) and volatile fatty acids, released as a result of microbial metabolism and autolysis (Briggs & Kear 1993). In order for precipitation of calcium phosphate to occur, the pH must drop below the carbonic acid dissociation constant (pH 6.38); passing this threshold is known as the calcium carbonate/phosphate 'switch' (Briggs & Wilby 1996).

Previous studies on coleoid taphonomy have posited that preservation potential may be related to post-mortem buoyancy (Kear *et al.* 1995), however, we suggest that *in vivo* buoyancy regulatory methods directly affect preservation potential. Coleoids can be broadly divided into two distinct groups: the negatively and neutrally buoyant (Clarke *et al.* 1979). The negatively buoyant coleoids are much denser than water and must constantly create dynamic lift via active swimming in order to maintain their position in the water column (Denton 1974). Often, these cephalopods are typified by the muscular pelagic squid and the Incirrina octopods (e.g. the 'classic' octopus). These octopods are typically benthic organisms and do not use chemical buoyancy methods.

Neutrally buoyant coleoids employ four main strategies in order to maintain their vertical position within the water column: gas-filled shells (found in cuttlefish and *Spirula spirula*); storage of low density fats within digestive glands (Gonatidae family of squid); substitution of sulphate ions with chloride ions within body tissues as well as extremely high tissue water content (~95% B. A. Seibel, pers. comm. 2013; seen in *Vampyroteuthis* and the Cirrina octopods); and finally, the accumulation of ammonium chloride ions to create low density fluids (Clarke *et al.* 1979). The last method, of creating ammonia-rich fluid, is the most prevalent in extant cephalopods (Clarke *et al.* 1979). It is particularly prominent amongst the squid that inhabit deep water, where the requirement for locomotion is decreased and the relative energetic expenditure of maintaining buoyancy is diminished by using chemical rather than muscular means (Seibel *et al.* 2004).

Ammonia is readily available to cephalopods as a result of a carnivorous diet (Seibel *et al.* 2004) and is the principle end product of their nitrogen metabolism (Boucher-Rodoni & Mangold 1995). High concentrations of ammonia are toxic at the cellular level (Pörtner & Zielinski 1998) and consequently, the Cranchiidae (the glass squid) have uniquely evolved a large specialized coelomic chamber for storage of ammonia fluids (Denton *et al.* 1969). However, the vast majority of neutrally buoyant teuthids do not possess specialist storage organs and sequester the fluid through muscular tissue in both mantle and arms (Seibel *et al.* 2004) in specialized vacuoles (Pörtner & Zielinski 1998). Ammonia is only marginally lighter than sodium and therefore vast quantities are required to make the animal less dense than the surrounding seawater (Clarke *et al.* 1979). Over 50–60% of the body mass of ammoniacal teuthids is ammonia fluid (Clarke *et al.* 1979; Voight *et al.* 1995; Seibel *et al.* 2004).

Using the Cretaceous Lebanese Lagerstätten of Hâkel and Hâdjoula as a case study, we propose that a previously unknown taphonomic bias inhibits teuthid tissues from generating the required pH threshold for phosphatic preservation and may explain the lack of teuthid coleoids in the fossil record. We hypothesize that the differing modes of life and the varying methods of buoyancy control between the vampyropod and decabrachian groups have a direct impact on the probability of soft tissue preservation.

Institutional abbreviations. LEIUG, University of Leicester Department of Geology, UK; NHMUK, Natural History Museum, London, UK.

MATERIAL AND METHOD

Coleoid and fish material was obtained from a local fish merchant based in Bristol, UK. Twenty deceased incirrate octopod (*Octopus vulgaris*) and twenty decabrachian loliginid squid (*Loligo vulgaris*) specimens were selected and purchased due to their sustainable fishing stocks and seasonal availability within UK waters. These animals represent the two distinct coleoid groups and can be used for comparative decay analysis. Three species of fish, *Melanogrammus aeglefinus* (haddock), *Sprattus sprattus* (European sprat) and *Scomber scombrus* (Atlantic mackerel), were also acquired. Fish belonging to the same clade as the extant sprat family, such as *Diplomystus* and *Scombroclupea* (extinct genera of Clupeiformes), have been found with phosphatized tissue in the Hâkel Lagerstätte, Lebanon, alongside examples of Vampyropoda (Woodward 1896; Hay & Day 1903). As we observe many genera of fishes exhibiting soft tissue preservation within Lagerstätten formations worldwide, they are ideal animals for comparative decay investigation. All animals were mature adults which met EU size regulations and were undamaged during retrieval. The animals were trawled the evening prior to being purchased and were subsequently kept on ice for approximately 6–7 h before retrieval. Dissection was carried out immediately.

Paired samples were taken mediolaterally from the left and right ventral mantle of both octopus and squid, taking care to keep the dermis as complete as possible. The wet mass of these samples was measured (avg. *L. vulgaris* mantle = 18.10 g, n = 22; avg. *O. vulgaris mantle* = 20.03 g, n = 22). An arm was also sampled from the octopus (taken from the first arm pair) (avg. weight = 28.66 g, n = 11). As the arms and tentacles of *L. vulgaris* have little mass, the heads and all the limbs were utilized as per Kear *et al.* (1995) (avg. weight = 24.93 g, n = 11). These samples were taken from the area anterior to the eye capsules, and the buccal mass was carefully removed. Each sample was placed in a sealable glass jar with 250 ml of artificial seawater (ASW) (pH = 8.01 ±0.03). A haddock was also dissected and two tissue samples were taken from the region posterior to the pectoral fin, mediolaterally, but anterior to the anal fin, below the lateral line, ensuring that the dermis and scales remained intact. Any large bones were removed (avg. weight = 16.65 g, n = 2).

Nine squid and octopod carcasses were kept intact and used for whole body experiments for reference and morphological comparison. These carcasses were kept in non-sealed tanks containing 2500 ml ASW. The Atlantic mackerel and sprat were also kept as whole body experiments in 2500 ml ASW tanks (82.91 g).

The ASW was made by adding seawater minerals ('Tropic Marin') to distilled water in the instructed amounts: 1 kg per 25 l (salinity = 33–36 ppm) and allowed to dissolve for 24 h. The jars were sealed and placed in an incubator set at 20°C, so as to retard the rate of decay (Kidwell & Baumiller 1990). No external bacterial inoculum was added to the water, as repeated opening of the jars and the carcasses' indigenous flora were sufficient to cultivate a microbial population (Briggs & Kear 1993). The samples settled for 24 h before the first pH reading, which was consequently taken twice daily using a METTLER TOLEDO SevenGO SG2 pH meter (reading accuracy ±0.03) and morphological descriptions were recorded. The average ASW starting pH for all experimental runs was 7.63 (±0.03).

Jars were removed from the incubator, allowed to settle for 30 min, a reading was taken and then immediately returned to the incubator; taking care to disturb the samples as little as possible. Repeated opening of the jars allowed the water to remain oxygenated and did not impede decay rates (Briggs & Kear 1993). The pH readings were taken repeatedly from a pre-determined location and depth in the jars (medially between the tissue and jar) and carcass tanks (along a parallel axis to the carcass running from the apex of the mantle, head region and arm section respectively) to avoid producing any false pH gradient readings. The experiment was conducted for three consecutive runs of 14 days and any remaining tissues were decanted and water samples appropriated for chemical analysis.

A Hanna HI 96700 Ammonia Low Range ISM was used to analyse the ammonia-nitrogen (NH3-N) content of the ASW once the tissues had been submerged for 24 h, and then on a weekly basis until termination. The HI 96700 uses photometric chemical analysis to calculate the amount of ammonia-nitrogen present by passing light of limited spectral bandwidth through a clear cuvette filled with 10 ml of the sample liquid and then through a narrow band interference filter (420 nm). The light is then detected by a silicon photocell. After zeroing each sample, Hanna reagent is added (six drops of Hanna HI 93700A-0 and 10 drops of HI 93700B-0). The reaction between the reagents and the ammonia causes a yellow tinting effect, created by formation of an 'absorbing compound', causing a fraction of the emitted light radiation to be absorbed. The amount of light absorbed allows the molar concentration of ammonia-nitrogen present in the water to be calculated. To ascertain the ammonia concentration (mg/l) the original reading is multiplied by a factor of 1.214. This method is an adaptation of the D1426-92 Nessler method and the instrument is accurate to ±0.04 mg/l at 25°C. In order to ascertain a reading within the instrument's range of 3.00 mg/l NH3-N, 1 ml of ASW was extracted from the sample jars (ensuring no tissues were collected) and diluted with 9 ml of standard ASW (i.e. ASW from the batch pre-mixed prior to filling the jars and tanks with tissues) and a reading taken. This was then scaled appropriately to give final measurements.

RESULTS

Whole body experiments

The whole body experiment showed that during the 14 observed days, the squid carcasses did not reach a pH low enough to enter the calcium phosphate window (Fig. 3). Conversely, the octopus carcasses reached the threshold for calcium phosphate precipitation within 4 days and remained below pH 6.38 until after the termination of the experiment.

The whole body fish experiments validated the hypothesis that fish carcasses can generate the required pH for authigenic calcium phosphate precipitation. The mackerel pH dropped extremely rapidly but crossed the pH threshold at the same time as the octopus carcass, and generated a pH below 6.38 for the rest of the experiment. The sprat's pH was significantly lower by Day 1 than either of the coleoids or the mackerel. Its pH trajectory slowly rose until Day 10 when it passed the 6.38 boundary and did not fall below it again for the duration of the experiment. The rise in pH on Day 8 coincided with the sprat carcass floating and Day 12 marked the rupture of the animal's abdominal cavity, spilling what remained of the internal organs into the ASW.

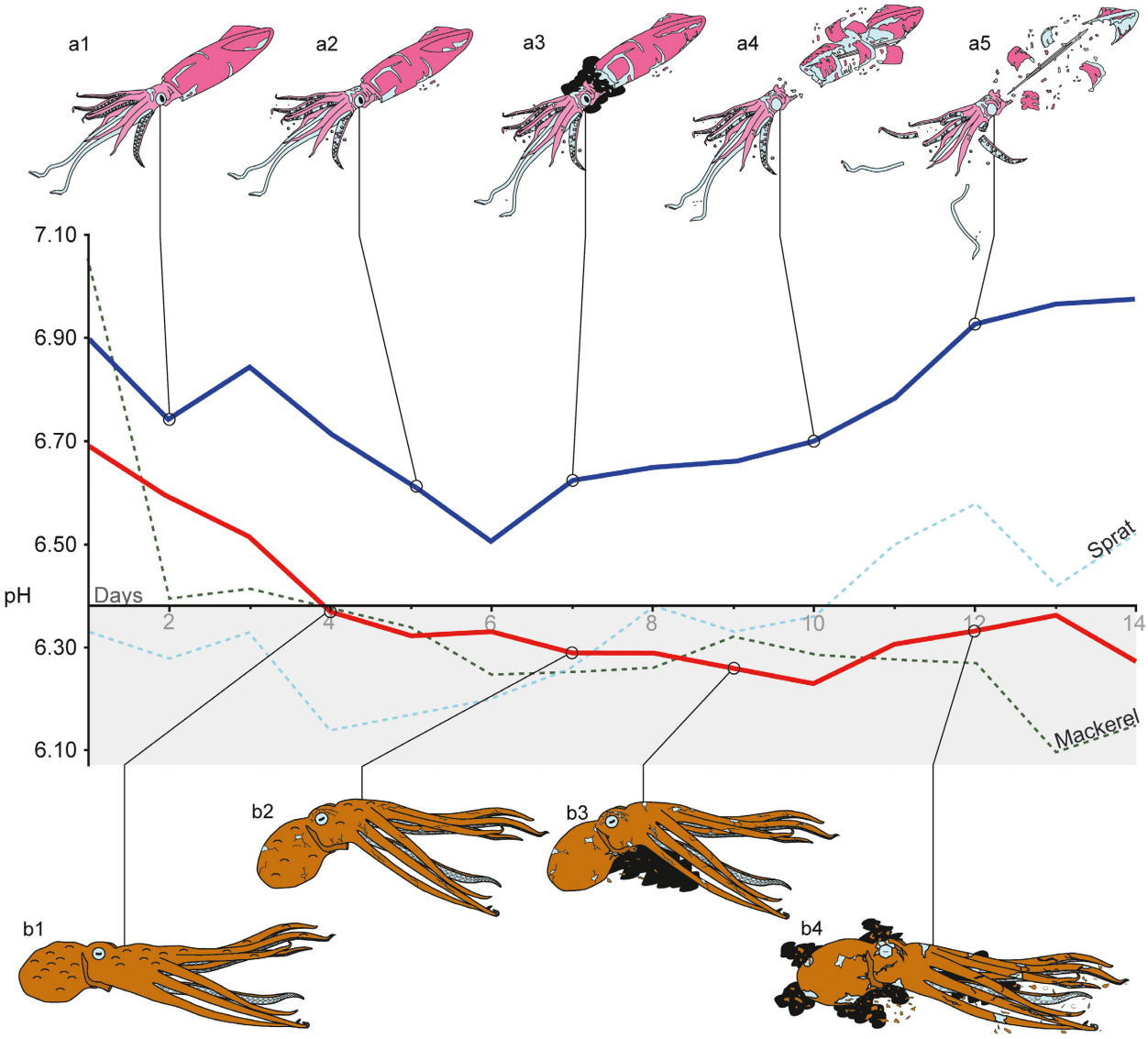

FIG. 3. Decay and pH trajectories of whole body *Loligo* (a1–a5), *Octopus* (b1–b4) and fish carcasses over the 14 days. The light grey area indicates the pH window for calcium phosphate precipitation (below 6.38). The coleoid data is averaged over three experimental runs. The average range for octopus carcasses was 0.26 and for squid carcasses, 0.30. All three experimental runs for both carcasses can be seen in Clements *et al.* (2016). Colour online.

Mantle

During the initial 24 h there was a significant drop in pH of the ASW around both octopus and squid mantle samples. However, the octopus mantle fluid was more acidic than the squid (Fig. 4). The octopus mantle entered the phosphate precipitation window of 6.38 by Day 3 and remained inside the window until the termination of the experiment. In contrast, the squid tissues did not enter the phosphate precipitation window for the whole duration of the experiment. In both tissue samples the pH began to plateau around Day 5, although there was an anomalous pH spike during the sixth day. In contrast to the cephalopod tissues, the pH of the haddock tissue sample plummeted to within the phosphate window in 2 days. It remained below the 6.38 threshold until Day 10, although the pH did not fall as low as that generated by the decaying octopus tissues. Although the haddock tissue pH did exceed 6.38, it remained very close to the threshold for the remainder of the experiment.

Arms

In both the squid and octopus arms there was also a rapid decrease in pH during the first 24 h, although the pH of the ASW surrounding the octopus arms was relatively higher than that of the octopus mantle during this time; inversely, the squid arms had a lower pH than their mantle equivalent (Fig. 4). The octopus arms entered the

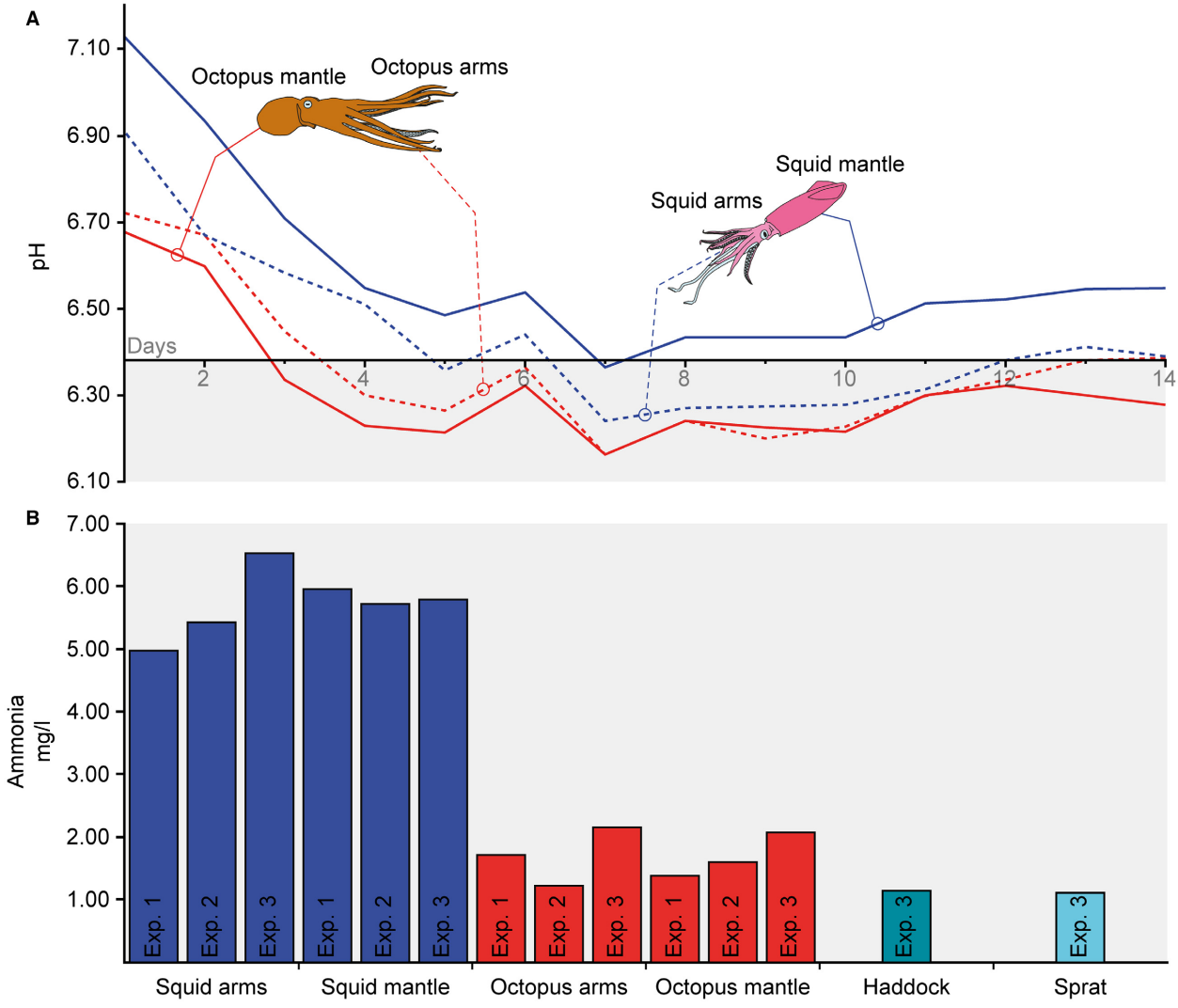

FIG. 4. A, pH trajectories of coleoid mantle (dotted lines) and arm (solid line) tissues over 14 days; the data is averaged over three experimental runs; the light grey area indicates the pH window for calcium phosphate precipitation (below 6.38); average ranges: squid arms 0.22, squid mantle 0.21, octopus mantle 0.31, octopus arms: 0.17. B, ammonia content in the fluid surrounding cephalopod and fish tissues at the end of each experiment. Colour online.

pH window suitable for phosphate precipitation by Day 3 and remained below the threshold until Day 13. The squid arm pH decreased and passed the threshold on Day 5, albeit only for 24 h, re-entering the precipitation window on Day 7. Between the seventh and ninth days, the pH in the arm tissues of both taxa began to rise toward leaving the phosphate window, however the squid arms breached the threshold first on Day 12, while the octopus tissues following the day after. The anomalous spike in pH seen in the mantle tissues on Day 6 is mirrored in the arm tissues, suggesting either a taphonomic phenomenon or a recording artefact.

Decay stages and character loss

The tissue samples kept in the jars showed a significant difference in decay during the experiment when compared with the whole carcasses (Fig. 3). The squid mantle tissues often resisted decay for the entirety of the experiment, whereas the octopus tissues often disintegrated by days 9–14. This is also the case of the arms, which would survive the experiment in the squid samples, although severely bloated and swollen due to osmotic effects, while the octopus arms were significantly decayed by Day 11 and almost completely disintegrated by Day 14. The ASW within the jars with the octopus samples turned a milky red colour, while the liquid accompanying the squid tissues turned milky white with a thick yellow immiscible scum layer at the surface that had a distinctive sickly sweet smell.

During the whole body experiments, the octopus carcasses remained more or less intact, whereas the squid disarticulated (see Fig. 3a1–5, b1–b4). The dermis of the squid carcasses began to rupture and split by Day 2 (Fig. 3a1). The carcasses often bloated and began to float to the surface by Day 3 (although not all carcasses floated) when the first loss of suckers occurred. Within five days, they became extremely fragile, friable and susceptible to full disarticulation with the most minor disturbance (Fig. 3a2). Around Day 7, the head often separated from the mantle, and internal organs such as the ink sack spilled their contents into the surrounding fluid (Fig. 3a3) giving off a distinctive sickly sweet smell. By Day 10, the mantle became very gelatinous and was ruptured by the gladius (Fig. 3a4). Over the next two days, the mantle fully disarticulated and the arms began to separate from the head, although the arm crown always clearly showed stumps of ten arms (Fig. 3a5). The first visual sign of decay in the octopus carcasses was around Day 4 when the skin began to 'bubble' (Fig. 3b1). These bubbles began to split by Day 7, causing the dermis to peel away in small areas, exposing the muscle tissue (Fig. 3b2). By Day 9, the water around the octopus carcass began to discolour and turn black, accompanied by an obnoxious smell, indicating that the ink sac and internal organs had ruptured (Fig. 3b3). By Day 12, the mantle began to rupture and degrade, especially around areas where the dermis had previously split, and the first suckers began to disarticulate, though the animals remained fairly well articulated (Fig. 3b4).

Ammonia

All three experiments demonstrated that the ASW surrounding the squid tissues had more than double the amount of ammonia measured around the respective octopus tissues, although both coleoids had increased ammonia content compared to the two fish taxa (Fig. 4). The ammonia content of the squid arms was not always more than that of their mantle tissues, which does not corroborate our current understanding of ammonia sequestration within squid tissues (i.e. Lipiński & Turoboyski 1983; Seibel *et al.* 2004).

DISCUSSION

Calcium carbonate obliterates the ultrastructure of labile tissues (Allison 1988*a*). Therefore, to obtain the high morphological fidelity seen in the fossil record of the Hâkel and Hâdjoula Lagerstätten, calcium phosphate must have replaced these tissues. The oceanic marine environments that coleoids inhabit are supersaturated with calcium phosphate. Nevertheless, precipitation is impeded by high levels of oceanic bicarbonate ions and kinetic factors (Briggs & Wilby 1996). As a result, calcium carbonate preferentially precipitates unless the local pH is reduced below the calcium carbonate/phosphate switch of 6.38 (Allison 1988*a, b, c*; Briggs & Wilby 1996). The generation of a low pH via decay has been cited as one of the most important contributing factors of the phosphatization process (Briggs & Wilby 1996).

Our data show that, assuming the coleoid carcasses are rapidly entombed in a microenvironment that is 'closed' (i.e. the diffusion of decay products away from the carcass is restricted), the decay of octopod tissues can sufficiently lower the pH of the surrounding pore water to allow calcium phosphate to precipitate and replace labile tissues. Conversely, the likelihood of decabrachian tissues being replaced by calcium phosphate, in environments conducive to coleoid soft tissue preservation, is compromised by the inability to generate a low enough pH.

This suggests that the disparity in preservation potential between coleoid groups is caused by the differing modes of buoyancy regulation. Neutrally buoyant teuthids

utilize the waste product of their metabolism, ammonia, as a buoyancy aid because it is lighter than water. Ammonia acts as a buffer to the pH microenvironment created by decay of the carcass and prevents phosphate replacement of soft tissues. However, teuthids such as *Loligo vulgaris*, used in this experiment, are negatively buoyant and must actively swim to maintain their position in the water column. Our data demonstrate that the ammonia content of negatively buoyant teuthid tissues is very high, indicating that squid that do not use ammonia as their primary buoyancy method still have sufficient quantities of this chemical present in their tissues to inhibit their preservation via phosphatic replacement. Our findings corroborate previous studies (e.g. Boucher-Rodoni & Mangold 1995) that demonstrate that negatively buoyant squid (e.g. *Loligo forbesi*) excrete more than twice the amount of ammonia that cuttlefish or *Octopus vulgaris* do, which is likely to relate to the high metabolic cost of active swimming.

Briggs & Wilby (1996) observed that the decay of carcasses is 'dynamic' and localized microenvironments are created by differing parts of the anatomy during decay. Our experiments show no evidence of large-scale pH variations along the axis of teuthid carcasses, suggesting that the ammonia was not concentrated in a specific area of the anatomy but rather throughout the animal, further limiting its preservation potential. Kear *et al.* (1995) did not observe any phosphatic precipitate within the soft tissues of a teuthid that had been decaying for 4 weeks in conditions supersaturated with calcium phosphate. Elevated ammonia levels may have buffered this carcass during that experiment. Therefore, it is highly likely that high levels of ammonia found within teuthid soft tissues, compared to those of octopods, impede their chances of ending up in the fossil record.

Furthermore, our experiments showed that the robustness of octopod carcasses explains the greater likelihood of preservation compared to decabrachian squid carcasses. One biological factor that is commonly attributed to high fidelity replication of coleoid and fish soft tissues is how long the dermis keeps its integrity (Briggs 2003). In our experiments, the teuthid dermis split and decayed in the first 24–48 h, as seen in previous studies of coleoid decay (Kear *et al.* 1995), whereas the octopus dermis began to lose integrity between days 7 and 9. The rupturing of the dermis allows underlying tissues, such as muscle, to be invaded by bacteria thus increasing the rate of decay. This explains why squid labile tissue degeneration is far more rapid than that of octopods, with muscle ultrastructure being lost within one to one and a half days (Kear *et al.* 1995). The size of the animal may also be a factor; the size of the carcass affects decay rate, with increased mass and decreased surface area impeding the diffusion of electron donors required for microbial anaerobic respiration (Allison 1988*c*). Due to their comparatively smaller size and increased surface area, the teuthid arm crown and tentacles decay rapidly, further decreasing the probability that the arm crown, the important distinguishing decabrachian character complex, will be replaced by diagenetic minerals.

Ancestral states of ammonia sequestration

It is unclear whether the use of ammonia as a buoyancy regulator is an ancestral state of the Decabrachia (Voight *et al.* 1995). Phylogenetic bracketing, however, indicates a high probability that elevated ammonia levels were used by the common ancestor. Stem decabrachians, such as the belemnoids, occasionally preserve phosphatized soft tissues although the fidelity of soft tissue preservation at the cellular level of these animals is often poor compared to that of the vampyropods (Fuchs *et al.* 2007). These animals used an internal chambered shell, the phragmocone, as a mode of buoyancy control. Fossil evidence indicates that these animals were active or moderate swimmers drifting in the water column and had a carnivorous lifestyle (Klug *et al.* 2016). This, coupled with a metabolism that is likely to have been nitrogen based, suggests that they too would have elevated levels of tissue ammonia. Although belemnoid coleoids have occasionally been reported from the Lebanonese Lägerstatten (Fuchs 2007), they are not as well-preserved as the vampyropods (Engeser & Reitner 1986). The fact that belemnoid soft tissues exist in the fossil record (Wilby *et al.* 2004) suggests that this was not enough to inhibit phosphatic replacement, however it may have reduced the fidelity of soft tissue preservation.

Kear *et al.* (1995) noted the morphological similarities between phosphatized muscle tissues of the stem decabrachian *Belemnotheutis* from the Jurassic, and extant decabrachians. Our experiments also showed that heavily decayed teuthid mantle often resembles the 'blocky' textures seen in phosphatized muscle tissues in belemnoids (Fig. 5). Previously, the cause of this texture in fossil material was posited to be sedimentary burden and subsequent taphonomic collapse and compression of the carcass (Allison 1988*b*; Wilby *et al.* 2004). However, our observations demonstrate that it may be the preservation of muscle tissue that has contracted due to decay.

Re-interpretation of ambiguous material

Understanding the taphonomic biases that effect the preservation of coleoids allows us to inform the current debate on the Vampyropoda hypothesis. One such controversy is that of the Jurassic coleoid fossil *Mastigophora*, which has been identified as a decabrachian teuthid since

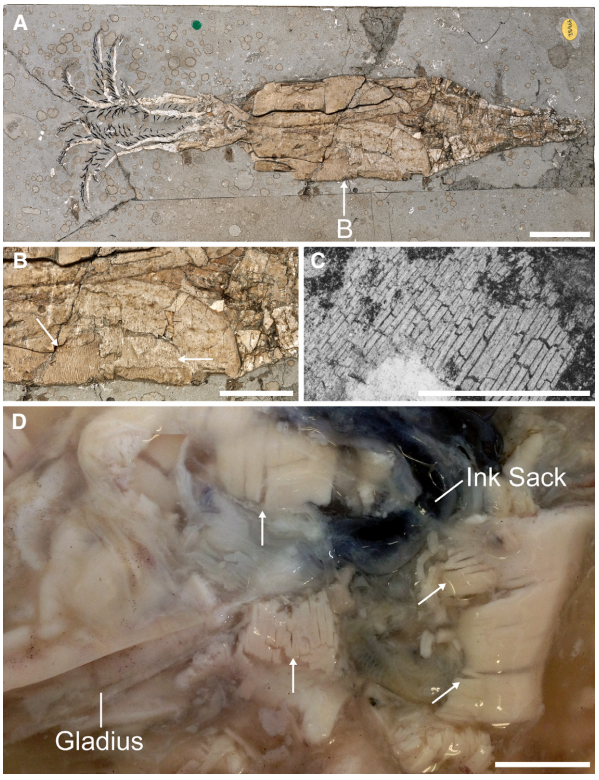

FIG. 5. Decay textures within stem decabrachians. A, *Belemnotheutis antiquus,* Christian Malford, Wiltshire, England, Callovian Oxford Clay, NHMUK 25966 with the area of B marked. B, magnified view of the 'blocky texture' seen in the mantle region, seen as striations on the tissue marked with arrows. C, *Belemnotheutis* muscle fibres showing 'blocky texture' LEIUG12814(1) Bed 2, Peterborough Member, Oxford Clay Formation, Wiltshire (reproduced with permission from Wilby *et al.* 2004). D, *Loligo* mantle tissue after 14 days of decay. The arrows indicate areas of muscle which has contracted and decayed creating 'blocky' textures. Scale bars represent: 2 cm (A, B, D) (Photos A and B courtesy of Jonathan Jackson, NHMUK); 1 cm (C). Colour online.

its first description by Owen in the nineteenth century (Owen 1856). Vecchione *et al.* (1999) concurred that it was a decabrachian having identified a 'thin filamentous extension of the arms', rejecting the suggestion that these could be homologous with vampyromorph filaments. When noting that not all *Mastigophora* fossils they examined had these filaments or even the presence of tentacle bases (the connection between the arm crown and tentacle), they stated that 'this could easily result from either preservation or preparation artefact' (Vecchione *et al.* 1999, p. 118). This view was shared by Young *et al.* (1998, p. 397): 'Soft parts are rarely preserved and, if they are, the absence of tentacles may simply be a preservation artefact; as a result, their presence can only be assumed.' Our experiments on the decay of crown group decabrachians demonstrate that the arm crown is one of the most robust parts of the carcass and remains fairly intact for much of the sequence of decay. Tentacles do disarticulate from the arm crown, but they always leave stumps, so that the number of appendages can still be ascertained. This suggests that the argument put forward that putative fossil teuthids could consistently lose their tentacles during fossilization (Young *et al.* 1998; Vecchione *et al.* 1999) is fairly weak, corroborating the findings of Fuchs *et al.* (2013*b*). Another argument suggests that tentacles held in tentacular pouches would not be preserved (Vecchione *et al.* 1999). We agree with Kear *et al.* (1995) that during decay, teuthid carcasses tend to be more buoyant toward the posterior, with the mantle higher than the head, causing tentacles to fall out of any pouch. These taphonomic factors make it highly unlikely that tentacles would be present in these ancestral coleoids but consistently not preserve. Fuchs (2014) made convincing counterarguments against the structures in *Mastigophora* being actual tentacles and if *Mastigophora* is a decabrachian from the Callovian, it appeared long before the Jurassic–Cretaceous divergence proposed in recent molecular clock studies (Kröger *et al.* 2011). We, therefore agree that the evidence suggests that *Mastigophora* is indeed a vampyromorph.

Two extant gladius-bearing decabrachian families, the *Lepidoteuthidae* and *Octopoteuthidae*, are known to reabsorb their tentacles during ontogeny after the paralarval and juvenile stages. The adult animals have arm crowns with eight arms and no 'stumps' from the lost tentacles. This adaptation is restricted to these groups; the fact that the larvae have ten arms supports that the theory that loss of the tentacles is a derived character state which would not be expected in the stem lineages (Donovan & Fuchs 2016). Furthermore, *Lepidoteuthidae* and *Octopoteuthidae* use ammonia as a buoyancy method (Clarke 1988) and so are unlikely to be preserved in the fossil record.

Further investigations

The decay of coleoid gladius characters was not part of this experiment, but an investigation of the preservation potential of cephalopod gladii is an avenue of further investigation which may help elucidate the inexplicable absence of decabrachian gladii in the fossil record.

This experiment also raises questions about the preservation potential of close relatives of the coleoids – the ammonoids (Ritterbush *et al.* 2014). The fossil record of ammonite soft tissues is severely limited and there are currently no known specimens that reveal the number of arms these cephalopods had (Ritterbush *et al.* 2014; Klug & Lehmann 2015; De Baets *et al.* 2016). There are several biostratinomical reasons which might limit the ammonoid fossil record (Ritterbush *et al.* 2014) for example, a build-up of gases in the soft tissues and chambered shell

may have caused the carcass to float post mortem, or high levels of body tissue ammonia may have reduced their preservation potential. Nautiloids, an extant relative of the ammonoids, also have a poor labile tissue fossil record (Mehl 1984). Studies have demonstrated that they too excrete considerable amounts of ammonia (Schipp & Martin 1981; Pernice *et al.* 2007) and therefore taphonomic experiments on the decay of *Nautilus* soft tissues may explain the poor fossil record of these important cephalopod groups. Unfortunately, their vulnerability to over-exploitation (Dunstan *et al.* 2011) makes this unfeasible at present. Interestingly, 'exceptionally' preserved ammonoids (Wippich & Lehmann 2004) and nautiloids (Keupp *et al.* 2016) from the Lebanese Lagerstätten are known with stomach contents and jaws *in situ*; indicative of rapid sinking and burial with soft-tissues in place (Wani 2007). Labile soft tissues seen in the Lebanese fossil vampyropods, such as arms, have so far not been reported.

Evaluation of experimental design

Our data show that using tissue samples, as opposed to carcasses, is not appropriate for taphonomic experiments. Before the experiment, it was believed that the greater surface area of the squid heads compared to octopus arms of the same weight might skew the decay trajectories but our experiments demonstrate the opposite. Bacterial invasion of the dissected octopus arm caused it to disintegrate much sooner than the equivalent weight of complete squid head used in the experiment. Therefore, it is likely that the open 'wound' caused by the dissection and the subsequent compromise of the dermis allowed greater bacterial invasion and a faster decay rate. The squid head samples show considerably greater decay compared to the complete carcasses by the experiment termination, although on Day 10 the head of the squid carcass did disarticulate in the whole body experiment at the same place that the dissection was made in the tissue samples. We therefore suggest that future taphonomic experiments based on dissected tissue samples are not a suitable proxy for gauging rates of decay and character loss.

CONCLUSION

A number of factors contribute to a taphonomic bias preventing gladius-bearing crown decabrachians from becoming fossilized, including a more fragile and faster decaying dermis, comparatively smaller carcass size, and a larger surface area than octopods. However, the predominant factor is the large amount of ammonia which is sequestered throughout the animal in both mantle and arm muscle tissues, which acts to buffer the pH around a decaying carcass and inhibits the precipitation of authigenic minerals such as calcium phosphate, impeding the replacement of soft tissues. These factors exclude gladius-bearing crown decabrachians from the fossil record.

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Author contributions. JV conceived the project. TC designed the experiments. TC and CC undertook the dissection and decay experiments. TC wrote the initial draft and CC, KDB and JV developed the manuscript. All authors reviewed and edited the final manuscript.

DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.12pn2

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